ESTABLISHMENT AND COMPETITIVE ABILITY OF *NELUMBO LUTEA* IN RELATION TO *MYRIOPHYLLUM SPICATUM*

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Limitations from reduced light and increasing water depth on *Nelumbo lutea* seedlings were determined in tank experiments. Survival was high in all tested light levels. Total biomass increased significantly with increasing light. Biomass allocation shifted significantly to root production between 3 and 6 weeks in the 10 and 24% levels. Survival decreased with increasing planting depth, and biomass of survivors reduced significantly between 0.5, 1.0, and 1.5 m depths.

Nelumbo lutea and *Myriophyllum spicatum* populations were monitored for one season in a 0.7 ha pond to track changes in species dominance. *Myriophyllum spicatum* dominated early, and *N. lutea* dominated from July through October, suppressing *M. spicatum* at all depths.

Competitive interactions between *N. lutea* and *M. spicatum* were investigated for two seasons in a container experiment situated within a pond. Where established, *N. lutea* dominated in the presence of *M. spicatum*. However, *N. lutea* could not be established in depths greater than 1 meter.

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CHAPTER 1.

INTRODUCTION

A diverse plant community characterizes a healthy aquatic ecosystem. Excessive growth of plants, however, can be detrimental to the function of that system. Native aquatic plant communities contribute to the productivity and overall health of aquatic habitats by providing food, shelter, sediment stabilization, excess nutrient removal and improved water clarity. (Madsen, 1993; Doyle and Smart, 1995). Non-native, invasive macrophytes, however, can degrade the quality of aquatic ecosystems by excessive growth throughout the water column and on the water surface. Exotic macrophytes also exhibit the capability of rapidly colonizing a disturbed area devoid of native aquatic plant populations, creating monocultures not conducive to diversity and ecosystem health (Doyle and Smart, 1993).

Myriophyllum spicatum is a non-native submersed aquatic plant from Eurasia, possibly introduced into North America in the 1880's (Reed, 1977). This species creates problems in many lakes and reservoirs, and may actually benefit from ecosystem disturbances caused by management efforts to control its excessive growth (Smith and Barko, 1990). It is known to rapidly colonize and adversely impact aquatic systems, sometimes suppressing the growth of native plants (Madsen et al, 1991). Madsen (1998) studied the decline of native species under the dense shade of *M. spicatum* canopies and

expressed the need for research into the ability of *M. spicatum* to competitively exclude other species.

Nelumbo lutea is a native aquatic floating leaved and emergent plant, considered a keystone species in wetland systems (DeGroft and Francko, 1996). *Nelumbo lutea* is an important contributor to primary production in an aquatic system (Francko and Whyte, 1995) and serves as a source of food and shelter for wildlife (Whitley, et. al. 1990). It can rapidly colonize when propagules are introduced into an area, and its large, floating and emergent leaves create shade on the water surface (Doyle and Smart, 1995). Unlike *M. spicatum*, *N. lutea* does not occupy the water column with rank vegetative growth, but leaves an open area that facilitates gas exchange and allows other species to co-exist. For example, although *N. lutea* is abundant in Caddo Lake, Texas, in open cypress swamp areas, it co-exists among a diverse community of other native (and non-native) plant species (Van Kley, Hine, 1998).

The rapid growth of *N. lutea* petioles through the water column into a favorable light zone, and the apparent role of the petiole in photosynthesis in a developing *N. lutea* plant (Al-Hamdani, Francko, 1992) may suggest competitive advantages for early growth of *N. lutea* in adverse conditions as found when a dense canopy of *M. spicatum* is present. *Nelumbo lutea* was observed to have potential value in controlling noxious submersed exotics as early as 1971 (Smith, 1971) and more recently by research conducted in Guntersville reservoir, Alabama (Doyle and Smart, 1995).

The relatively high light requirement of *M. spicatum* (Madsen, 1991) could suggest that *N. lutea* may exhibit a competitive advantage for exploiting light resources by rapidly producing large, shade producing leaves. Madsen (1991) also indicates that, relative to *M. spicatum*, other native aquatic plants are shade tolerant and thus may not be negatively impacted under a canopy of *N. lutea* leaves.

The role of aquatic plant competition as it relates to community level dynamics of aquatic ecosystems has been discussed (McCreary, 1991) and competitive interactions play an important role in the structure of that community. Greater understanding of the effects of *N. lutea* and *M. spicatum* in a competitive setting could demonstrate the potential for using the deliberate introduction of *N. lutea* to either suppress an existing population of *M. spicatum*, or repress the ability of *M. spicatum* to invade.

Aquatic plant community manipulation (based on sound biological and ecological principles) as a management strategy to improve diversity and habitat value has been presented as an attainable goal (Nichols, 1991). Aquatic habitat restoration has grown as an area of research and field testing in efforts to improve habitat diversity and water quality, fish habitat, and resistance against non-native plant dominance in aquatic systems. (Doyle and Smart, 1995; Smart, et al. 1996). By reducing the availability of light and sediment nutrients, established *N. lutea* may contribute to a reduction in the potential for problems caused by *M. spicatum* (Smart and Doyle, 1998).

An investigation using *N. lutea* as a competitor against *M. spicatum* as a management strategy requires an understanding of successful planting and early establishment techniques (and limitations) of *N. lutea* to ensure successful introduction of

the species. Germination techniques have been studied (Snow, unpublished). Further investigations of *N. lutea* establishment examine light and depth limitations on the establishment of young transplants to develop guidelines for planting *N. lutea*.

Competitive interactions among native and exotic macrophytes have been investigated in Guntersville Reservoir, Alabama as part of the Aquatic Plant Control Research Program. Doyle and Smart (1995) found *Nelumbo lutea* was able to out-compete *M. spicatum*, with light inhibition of *M. spicatum* growth a likely factor for the suppression of *M. spicatum* invasion in an established *N. lutea* colony.

Competitive interactions were investigated when both species concurrently established within a pond. By rigorously studying depth and light limitations on the establishment of *N. lutea*, and observing an expanding established community of *N. lutea* and *M. spicatum* growing together, this research augments the work done by Doyle and Smart.

A series of studies was designed to investigate the competitive interactions between *N. lutea* and *M. spicatum*, and explore techniques to introduce *N. lutea* populations as an ecosystem management strategy.

Objectives

Three research objectives are presented below:

A. Determine establishment techniques for *N. lutea* seedlings (light and depth limitations).

B. Observe community dynamics of *N. lutea* and *M. spicatum* in a pond system.

C. Study competitive effects over two growing seasons between *N. lutea* and *M. spicatum* in an experimental setting.

The objectives described above were investigated in three experimental settings. The three studies are presented separately, with an overall discussion of findings. These studies were conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF), in Lewisville, Texas.

CHAPTER 2.

ESTABLISHMENT TECHNIQUES FOR *N. LUTEA*

Nelumbo lutea seedlings were grown for 6 weeks under various depth and incident light regimes from 07 September to 18 October 1994 to determine the impact of reduced incident light and increasing planting depth on seedling survival and establishment. In the light reduction study, half of the plants were harvested after 3 weeks of growth on 29 September. Short-term survival and growth of *N. lutea* was compared using above and below ground plant biomass measurements, and petiole and leaf counts and measurements.

Methods

Seed germination

Nelumbo lutea seeds, collected from the LAERF were physically scarified and germinated in distilled water. Single seedlings were planted in 2.5 L plastic pots filled with pond sediment and placed in a shallow fiberglass tank. After a two-week establishment period in early September 1994, to ensure short-term survival of seedlings, unhealthy plants were culled and the remainder was then randomly assigned to various

light and depth experimental treatments. The experimental tanks were filled with aluminum sulfate treated water from Lewisville Lake (alkalinity>50mg/L $CaCO_3$, total phosphorus<0.02mg/L). Six pots were harvested to quantify initial *N. lutea* biomass. After drying plants at 55^o to constant weight, biomass measurements were taken and analyzed for statistical normality with the Shapiro-Wilk statistic (Zar, 1984).

Light Effect at Constant Planting Depth

In this experiment I examined the impact of reducing incident light on seedlings growing at a constant depth. Twelve pots containing *N. lutea* seedlings were placed in each of nine 2000 L (1.8 m diameter by 0.75 m depth) fiberglass tanks. Water depth was maintained at 0.45 m above the pot surface. Triplicate tanks were used for each of three shading treatments of 24%, 10%, and 7% of incident light.

The tanks were situated under 50% polypropylene shade fabric and by random assignment, tanks were covered with additional layers of shade fabric to further reduce light levels. A Licor™ flat quantum sensor was used to determine incident light as photosynthetically active radiation (PAR, 400-700nm), then applying combinations of shade fabric to reduce available light. At the end of the first week of growth, light levels were again verified outside the shaded area and at the level of the pots inside each experimental tank.

Six *N. lutea* plants from each tank were harvested and processed after three weeks. After six weeks, the remaining six plants from each tank were harvested. At harvest, petioles were counted and leaf diameters were measured. Sediments were

washed from the roots and all plant material was dried at 55^{o} to constant weight. Dry biomass measurements were made separately on below ground and above ground material.

Above and below ground and total biomass measurements from the third week harvest were compared to the sixth week harvest using t-tests to determine differences in plant development over the study period. Mean numbers of petioles and mean shoot/root ratios were compared using analysis of variance between treatments from the final harvest.

Effect of Planting Depth

In this experiment I investigated the impact of increased depth on the survival and growth of *N. lutea* seedlings. Plants were grown in full sun. The planted pots were placed in six 14,000 L (2.5 m diameter by 3 m depth) fiberglass tanks. Eight plants were randomly placed on platforms suspended at the appropriate depths, to achieve target depth treatments from the sediment surface of the planted pots to the water surface. By random assignment, two tanks held plants at 0.5 m under the water surface, two tanks at 1.0 m, and two tanks at 1.5 m. During the growing period, water levels were maintained, and water temperatures near the surface were monitored each morning.

Temperature and light were monitored throughout the study. The surface water temperature ranged from 17.8^{o} to 25.8^{o} during the study period, with a mean temperature of 23^{o}. Photosynthetically active radiation was measured with a Licor™ flat Quantum

sensor just beneath the water surface and at the soil level of the pots at each depth treatment one week after moving the plants into the tanks.

Results

Initial samples were normally distributed (alpha= 0.05, p=0.83, Shapiro Wilk), with mean mass of 0.31g (± .02), indicating homogeneity in propagules used in the studies. Mean petiole numbers were 2.5 (± 0.2), and the mean petiole lengths were 30.6cm (± 3.8).

Light Effect at Constant Planting Depth

When comparing biomass allocation of plants harvested after three weeks growth to those that grew for six weeks, differences in characteristics of growth and total production were noted between treatments (Figure 1). After three weeks, above ground growth was well developed, and changed little between three and six weeks in the 10%, and 24% light treatments, but decreased significantly in the 7% treatment (t-test, $p<0.01$). Root growth significantly dominated the biomass increase in all treatments between three and six weeks (t-tests, $p<0.01$). Under 7% incident light levels, plants allocated three times as much mass to shoots as roots. Total biomass increased significantly between three and six weeks in the 10% and 24% treatments, but showed no significant growth in the 7% treatment (t-test, $p<0.01$).

Shade fabric reduced available light in all treatments, but plants grown during the same period in the nearby depth study were not shaded. The 0.5 m depth treatment (100% available light) was incorporated as a reasonable comparison to the reduced-light plants grown at 0.45 m depth.

Survival, petiole numbers, and biomass allocation were compared (Table 1). Plant survival was at or near 100% in all treatments. Under 7% incident light levels, plants produced significantly fewer petioles than plants grown under higher light regimes (ANOVA, $p<0.01$). The shoot: root ratio decreased significantly between all treatments (Kruskal-Wallis non-parametric ANOVA, $p<0.01$) with plants growing in higher light investing proportionally more mass to root tissues.

Average total biomass of *N. lutea* seedlings increased significantly with increasing light availability (Figure 2). Plants grown with 100% light available at the water surface produced more than 6 times the total biomass than those grown under 7% light (6.58 g to 0.98 g, respectively).

Effect of Planting Depth

Nelumbo lutea seedling survival was highly contingent on planting depth (Table 2). At the end of six weeks, *N. lutea* seedlings exhibited 100% survival at 0.5 m depth, and 56% at 1.0 m, but plant survival was reduced to 12.5% at 1.5 m depth. The number of petioles produced by the surviving plants was not significantly different due to variable numbers of survivors. The allocation of biomass was significantly different

between all treatments (ANOVA, $p<0.01$). Shoot: root ratios increased from 0.65 at 0.5 m to 7.94 at 1.5 m.

The total biomass production and allocation of biomass to aboveground versus belowground biomass of surviving plants was also strongly influenced by planting depth (Figure 3). Total biomass production at the end of six weeks significantly decreased with increasing depth (ANOVA, alpha=0.05). Total dry biomass of surviving plants when grown at 0.5 meters averaged 6.58 grams; 3.35 grams at 1.0 meter; 1.32 grams at 1.5 meters.

Above ground biomass of plants grown at 0.5 m was significantly greater than those grown at 1.0 and 1.5 m depths. Below ground biomass followed the same statistical trend.

Discussion

Results of investigations of effects of planting depth of *N. lutea* seeds indicate strong depth constraints for seedling establishment. Mortality of seedlings is high when planted in deep water (>1m). The few plants that survived from deep plantings showed weak above ground growth and very poorly developed roots. The power of traditional statistical analyses is weakened by the variability inherent when sample size decreases by mortality. The biological significance is implicit, however, as surviving plants grown at 1.0 meter or deeper exhibited successively weaker growth with increased depth. As developing petioles reach the surface and begin the process of energy production through

photosynthesis, expansion of root production follows. Without enough seed energy to get petioles to the surface and begin leaf production, plants cannot survive.

The vertical distribution of light as it relates to depth limitation of aquatic plant growth is a common assumption regarding plant distribution (Agami, 1980). Light limitations (from turbidity or depth) are unlikely to be the only major abiotic factor that limits survival and success of seedlings when conditions are otherwise favorable for growth. Under light-limiting shade fabric, elongating petioles and young leaves did not have a chance to receive full sunlight upon reaching the surface. Even so, in the light reduction treatments, survival was good at each light level. Comparing 3-week growth and 6-week growth, above and below ground biomass increased unless available light was less than 10%.

The percent PAR available at depth as compared to the surface was 41% for plants at 0.5m deep, 26% at one meter, and 19% at 1.5m deep (table 3). In the depth treatments, plants were subjected to an all-or-nothing situation. If petioles could reach the surface with enough energy reserve to produce leaves for photosynthesis and gas exchange, the plants would survive and grow. In depths at a meter or more, many of the plants could not make it to the favorable light climate at the surface, even though the available light at depth was equal to or greater than that found in the light-reduction treatments.

Although one might assume that reduced light availability in deeper waters would be a principal factor in the failure of young seedling survival, these data suggest otherwise. In the depth study, plants grown from 1.5 m depth showed very poor survival,

even though the average amount of light reaching the sediment surface of the pots (~19%) was virtually identical to the amount of light penetrating to the sediment surface in the shallower (0.45 m) 24% light tanks, where all plants survived (Table 3).

While establishment from seed is limited to shallow water, developing colonies of established *N. lutea* plants can rapidly expand into deeper water (Doyle and Smart, 1995). Large rhizomes from plants established in shallow water likely assist *N. lutea* colonizing into deeper water.

Turbidity, which was not studied in these experiments, also likely affects success of petiole and leaf production. Petioles are reported to function as photosynthetic structures, exhibiting reduced rates of elongation during low light conditions (Al-Hamdani and Francko, 1992). With limited energy available in seed endosperm, in many situations young *N. lutea* seedlings likely expend most of this energy in petiole elongation to the surface before more efficient photosynthesis from surface leaves can begin. This study suggests that *N. lutea* can successfully establish from seed even under unfavorable light conditions as long as the depth remains shallow (less than 0.5 m) until plants are well established. With anchorage furnished by well-developed roots, emergent leaves and strong rhizomes may then enable *N. lutea* to spread into deeper water. However, successful establishment is unlikely if seedlings are planted in deeper water.

The long-term survival of newly established *N. lutea* seedlings is likely to be greater in high light conditions. Plants initially invest energy in producing leaves at the water surface, and accelerated root production follows in conditions with adequate light. Inadequate light produces plants with reduced overall biomass, with greatly reduced

production of roots. Conditions with enough available light to allow plants to develop strong root systems would increase chances for long-term *N. lutea* success.

In natural settings, wind and wave turbulence are considered limiting factors for floating-leaved plants in general (Sculthorpe, 1967). The physical disturbance created by surface turbulence may uproot young plants that have not yet developed substantial roots. Young root systems could be a major factor limiting success of seedlings starting in depths greater than one meter. Doyle and Smart (1993) reported loss of *N. lutea* seedlings when planted at an average depth of 0.7 m, and observed uprooted young seedlings floating at the surface in one study site in Guntersville Reservoir, Alabama. Other factors that contribute to the relative survival and success of *N. lutea* seedlings deserve further investigation. It is apparent, however, that in heavy shade (under 10% full sun) and in depths of one meter or more, successful establishment of seedlings is poor.

Successful *N. lutea* establishment from seed is likely in full sun areas in shallow water. Although established *N. lutea* colonies expand rapidly and tolerate water level fluctuations, seedling survival is strongly dependent on shallow (<1m) water conditions.

CHAPTER 3.

COMMUNITY DYNAMICS

By observing the spatial distribution and species composition of plants in a pond dominated by *N. lutea* and *M. spicatum* over a growing season, I hoped to ascertain possible changes in dominance as the plant community developed (Madsen and Bloomfield, 1993). The data generated in this study were used to help determine if competitive interactions may likely be observed in a long-term experimental setting.

Methods:

Pond characteristics

The plant community dynamics in a 0.7 ha pond with an established population of *M. spicatum* and an early phase of colonization of *N. lutea* (planted into the pond from seedlings) was monitored using 6 permanent transects spaced on 20 meter intervals across the width of the pond.

The rectangular pond measured 59 m by 124 m (Smart, et al. 1995). The earthen banks sloped gradually to a maximum depth at 1.6 m near the western bank. Water for this pond was supplied by Lake Lewisville outflow, with some groundwater contribution from seepage. Water levels were kept constant during the study. The water clarity and light availability were good, comparable to other vegetated ponds at the LAERF (Smart,

et al. 1995). The sediment was predominately fine-textured, with relatively low organic matter content within a favorable range for aquatic plant growth (Barko and Smart, 1986).

Plant Community Characteristics

Native submersed macrophytes in the pond included the annuals *Chara vulgara* and *Najas guadalupensis* which grew from the sediment seedbank. *Potamogeton nodosus* and *Heteranthera dubia* were also present, and *Typha sp.* was the dominant emergent plant along the shoreline.

Nelumbo lutea seedlings had been planted in shallow water near the north shore of the pond in an attempt to establish a community of *N. lutea*. By late summer 1991, *Myriophyllum spicatum* had invaded the pond from an unknown vector, and was beginning to dominate the plant community.

By 1993, *Nelumbo lutea* had spread to cover a substantial portion of the water surface. The apparent mixed co-dominance by an aggressive native macrophyte (*N. lutea*) and a problem exotic macrophyte (*M. spicatum*) in the pond led to the observations during the 1994 growing season.

Monitoring techniques

Permanent transects were laid out at 20 meter intervals across the pond, by using a tape measure and installing t-posts at the shoreline (figure 4). Six transects were used to characterize plant presence by anchoring a polypropylene rope (marked at each 0.5m

interval) across each transect. Using a small flat-bottomed boat equipped with a clear acrylic box in front of the bow to view through the water column, point-intercept observations (Madsen and Bloomfield, 1993) were made at 0.5 meter intervals along each of the transects. Looking vertically through the water column, presence of all species observed were recorded at each transect position. The transect observations were repeated on five dates (May 06, June 01, July 29, October 05, and November 28) through the growing season of 1994.

During the first observation period, pond depths were measured at each interval along all transects, and these data were included to show presence or absence at specific depths. Pond depths ranged from 0.0 m at the shoreline to 1.6 m. Mean depth of all transect positions was 0.95 m, and mean depths of transects ranged from 0.67 m to 1.14 m.

Data analysis

The frequency distribution of species by date was compared to determine differences in abundance of *N. lutea* and *M. spicatum* at each sampling period. The occurrence of each species was figured as a percent of total number of possibilities of occurrence (expected frequency). Thus, the change in community dynamics of *N. lutea* and *M. spicatum* was noted for an active growing season.

The observations were then arranged into two depth blocks: plants growing in water less than 1.0 m, and those observed at 1.0 m or deeper. Differences in relative abundance by depth at each sampling period of the two dominant plants was recorded.

Results

Point-intercept observations were totaled for each sampling period, and the frequency of occurrence of plant presence was computed. Native submersed plants remained at less than one third of total plant presence; so further analysis was conducted on the dominant plants of interest (*N. lutea* and *M. spicatum*) to determine changes in their community structure.

Occurrence of *N. lutea* and *M. spicatum* was totaled for all transects each monitoring period and compared to the total number of transect positions available. The percent occurrence of each plant at each monitoring period was compared to note changes in frequency (figure 5). *Myriophyllum spicatum* was well established in May, 1994, with 65% occurrence overall compared to 26% for *N. lutea*. Coverage for *M. spicatum* had increased by June 1, decreased slightly by the end of July, then dropped to less than 10% coverage in October and November. Coverage for *N. lutea* increased at each monitoring period, dominating the pond with nearly 100% coverage in October. After a hard freeze, no living *N. lutea* was observed during the November observations.

Depth block analysis of the observations was used to elucidate differences in community composition by depth (figure 6). Early in the growing season, *M. spicatum* dominated in deeper areas (>1.0 m), but when *N. lutea* substantially expanded into these areas after July, it continued to expand while *M. spicatum* declined sharply.

Myriophyllum spicatum growing in the shallow regions of the pond (<1.0 m), was greatly suppressed in late summer. Also, in depths greater than 1.0 m, *M. spicatum* continued to decline through the November observations.

Nelumbo lutea expanded at all depths throughout the growing season. *Nelumbo lutea* achieved nearly 100% coverage at all but the shallowest and deepest depths by October.

Discussion

In this series of observations, *M. spicatum* coverage declined under an increasing canopy of *N. lutea* throughout the growing season. By October, *M. spicatum* exhibited marked inhibition when *N. lutea* coverage was greatest.

The decline in *Myriophyllum spicatum* at this time of year was not simply a result of normal phenological changes in the population. Observations of two adjacent similar sized *M. spicatum* research ponds during late summer and fall of 1994 indicated nearly full coverage in *M. spicatum*, with lesser components of *C. vulgara* and *N. guadalupensis* present at the same time the *M.spicatum* coverage in the study pond declined.

A study in two ponds adjacent to this study site in 1993 (one year prior to these data, but under similar environmental conditions), reported continued expansion of *M. spicatum* during summer and fall, although the rate of expansion was reduced (Madsen and Smith, 1997). Madsen and Smith (1997) also reported a slowing of growth of *M. spicatum* when water temperatures are above 25^{o}. *Nelumbo lutea* may exhibit competitive advantages that are maximized by introducing strong shading to *M. spicatum* during its period of slowed growth.

Conversely, Madsen and Smith (1997) note that in the southern United States, *M. spicatum* exhibits active growth after November (at the termination of these

observations). Therefore, a need exists to more closely examine the potential for competitive suppression by *N. lutea* of *M. spicatum* over a complete annual cycle, including the winter months not observed in this study.

Although it was shown that *N. lutea* seedlings might not successfully establish in depths greater than 1 meter (see above), second-year unrestrained *N. lutea* grew quite well in deeper water. *Myriophyllum spicatum*, which exhibited nearly 100% coverage early in the season in depths greater than 1 meter, was reduced to less than 1% coverage under the canopy of *N. lutea* by November.

Doyle and Smart (1995), reported incident light levels between 1 and 10% under a developed *N. lutea* canopy, and showed that in areas where *N. lutea* was abundant, *M. spicatum* biomass was significantly reduced. The findings in this study reinforce the findings of Doyle and Smart: where *N. lutea* is well established, *M. spicatum* is reduced.

CHAPTER 4.

LONG-TERM COMPETITION BETWEEN *N. LUTEA* AND *M. SPICATUM*

Competitive interactions between *N. lutea* and *M. spicatum* were investigated over two growing seasons in a container experiment set up in an earthen pond at the LAERF. The objectives of this study were to quantify percent cover of *N. lutea* and *M. spicatum* after being allowed to grow for two years to determine competitive effects.

Methods

Pond preparation and planting

In a drained 0.27 hectare earthen pond at the LAERF, the pond bottom was covered with woven textile barrier fabric, anchored with sections of steel reinforcing rods laid across the fabric to discourage unwanted plant growth from the pond seedbank. The grade of the pond was surveyed using a rod and transit to determine the depth profile that would result when the pond was re-flooded.

Forty 1.2-meter diameter by 0.20-meter height plastic wading pools were arranged on the pond bottom in 4 depth blocks to facilitate an experimental block design using depth as the blocking variable. Mean depths of the 4 depth blocks were:

0.8, 0.9, 1.0, and 1.2 meters. The water level in the pond was monitored and maintained using a standpipe and water additions when necessary.

Each of the plastic pools was partitioned into 7 equal planting areas (Figure 7). In early June, 1992, Lewisville Lake water was added to the pond, and as it covered pools, they were planted in a randomized arrangement of 10 different combinations of *N. lutea* and *M. spicatum* propagules arranged in each of the 4 depth blocks.

Nelumbo lutea seedlings that had been physically scarified, sprouted, and grown for 4 weeks in 5 cm peat pots in a greenhouse mesocosm tank were used as the *N. lutea* propagules. The *M. spicatum* propagules used were 10 cm apical tips harvested from a nearby research pond. Combinations of propagules were planted as follows in the plastic pools: *N. lutea*, *M. spicatum*: 1,0; 0,1; 6,0; 0,6; 7,0; 0,7; 3,4; 4,3; 1,6; 6,1.

Observations

During the first growing season (1992), propagules were replanted as necessary to maintain the combinations described above until plants were growing in all treatments. Observations were made periodically (July 92, September 92, January 93, June 93, and July 93) to determine relative percent cover of plants in each of the pools. A 1.5 m diameter polyethylene hoop was placed over each pool on the water surface. Percent cover was determined by looking down through the water column and visually assessing aereal coverage. Percent cover ranks (Bailey and Poulton, 1968) were determined for *N. lutea*, *M. spicatum*, or other (native submersed macrophytes). Percent cover ranks were as follows: 1=0-1%, 2=1-5%, 3=5-25%, 4=25-50%, 5=50-75%, 6=75-95%, 7=95-100%.

Data analysis

During the second summer of growth, when maximum biomass was attained (July 1993), observations were analyzed to determine competitive effects based on percent cover. Analysis included non-parametric one-way ANOVA (Kruskal-Wallis) to determine significant differences based on planting density of *N. lutea* or *M. spicatum*. A 3-way non-parametric ANOVA was used to determine significant differences based on number of *N. lutea* planted, the presence or absence of *M. spicatum*, and the depth block. SNK groupings were used to delineate significantly different groups (Zar, 1984).

Results

The number of *N. lutea* seedlings planted in pools had no significant effect on percent cover of *N. lutea* after two seasons of growth (alpha 0.05, p=0.14, nonparametric ANOVA on ranks). The presence of *M. spicatum* had no significant effect on percent cover of *N. lutea* (alpha 0.05, p=0.88, Kruskal-Wallis non-parametric ANOVA). *Nelumbo lutea* percent cover was, however, affected by the depth block (0.8m=0.9m > 1.0m=1.2m) (alpha=0.05, SNK) , showing a sharp decline in successful establishment at depths of 1.0 meter and greater (Figure 8).

Percent cover of *M. spicatum* was lowest in pools planted with *N. lutea* alone and highest in pools planted with *M. spicatum* alone (alpha 0.05, p=0.001, Mann-Whitney U-test) (Figure 9). Pools planted with both *M. spicatum* and *N. lutea* showed variable *M. spicatum* coverage ranging from very high to low *M. spicatum* coverage. No significant

difference in *M. spicatum* coverage was indicated due to depth block (alpha 0.05, SNK, 0.8m=0.9m=1.0m=1.2m) (figure 10).

Discussion

Nelumbo lutea exhibited the ability to become established regardless of the density of planting. The percent cover of *N. lutea* was greatly influenced by the depth in which it was planted. Seedlings grew quite well at depths under 1m, but failed to establish at depths greater than 1m.

Myriophyllum spicatum became established in all depth blocks, and spread to pools where it had not been planted, likely by migration of allofragments and autofragments (Madsen and Smith, 1997). Although *M. spicatum* was present in pools where it had not been planted, its percent cover remained under 50% in areas where only *N. lutea* had been planted. *Nelumbo lutea* exhibited competitive suppression of *M. spicatum* percent cover in areas where *N. lutea* became well established (Figure 11).

Myriophyllum spicatum often spreads by fragmentation (Madsen and Smith, 1997), and this mechanism of spread was probably not greatly hampered by the pools arranged in close proximity in this experimental setting. *Nelumbo lutea* however, likely increases its population size by rhizome movement outward, and the production of tubers at some distance from the original plant. This mechanism of plant spread was greatly hampered by the physical boundaries of the pools. Upon harvest for biomass sampling, tightly bound rhizome, tuber, and root masses were observed encircling the edges of the pools in which *N. lutea* had become established. In a setting where *N. lutea* spread is not

restrained, the plant may exhibit additional long-term competitive ability against *M. spicatum*.

CHAPTER 5.

DISCUSSION OF ALL FINDINGS:

Nelumbo lutea exhibited potential as a competitor species in relation to *M. spicatum*. Observations in an earthen pond with an established *M. spicatum* population impacted by a spreading colony of *N. lutea* indicated an overwhelming reduction in the occurrence of *M. spicatum* during a growing season. Floating and emergent leaves were abundant, creating a heavily shaded environment in the pond. It is likely that the shading effect caused by the *N. lutea* community was an important factor in the reduction of *M. spicatum* in the late summer. Warm summer water temperatures (>25 ^{o}) found in southern shallow water systems like this study pond create conditions that slow the expansion of *M. spicatum* (Madsen and Smith, 1997). Slower growth in warm-water conditions could enhance the effect of shade in suppressing the growth of *M. spicatum*. High light requirements of *M. spicatum*, coupled with higher respiration requirements in warm water may upset the daily carbon balance for *M. spicatum* resulting in plant decline (Madsen, et al. 1991). These findings reinforce those from observations in Guntersville Reservoir, Alabama by Doyle and Smart (1995). In that southern reservoir, similar reductions in *M. spicatum* were noted when well-established *N. lutea* plants expanded via stoloniferous growth in an area where *M. spicatum* was present.

The control exerted by *N. lutea* may be due to the expansion of this native plant at a time when *M. spicatum* was weakened. Madsen (1993) investigated control points in the phonological cycle of *M. spicatum* at LAERF. His results indicated that in late summer and fall, carbohydrate concentration as percent of dry biomass of root crowns and lower stems of *M. spicatum* was at a low point, and autofragment production was at a peak. *Myriophyllum spicatum* plants at this time have fewer reserves of carbohydrate to recover from a disturbance. Thus, *M. spicatum* may effectively be impacted during this part of the season by perturbation. Since *Nelumbo lutea* actively grows and expands during late summer and fall, the shade produced by its leaves further suppress weakened *M. spicatum* plants thereby facilitating its own expansion. During this active growth period, *N. lutea* is likely maximizing use of nutrient resources to the detriment of *M. spicatum* success.

As *M. spicatum* presence was reduced in deeper areas of the pond, it began slight increases in the shallowest areas late in the season. The spread of autofragments, and rooting and initialization of new plants may account for some of this late-season success.

The other native plants in this pond, though present in relatively small quantities, changed very little in species composition and abundance. It appears that other shade-tolerant native plant species may not be seriously impacted under a *N. lutea* canopy. Although a similar scenario in northern lakes may produce different results, evidence suggests strong competitive ability of *N. lutea* in relation to *M. spicatum* in southern aquatic systems.

In the longer-term experimental setting, the percent cover of *M. spicatum* was reduced by successfully established *N. lutea* when both plants became established concurrently and grew for two seasons. This setting was designed to investigate effects when a deliberate introduction of *N. lutea* might be used to reduce a population of *M. spicatum.* Successful establishment of *N. lutea* from seedlings was the key factor for competitive effects to be exhibited. Depth constraints for seedling establishment at depths around one meter and deeper severely limited the effectiveness of *N. lutea* as a competitive species.

Shade produced by *M. spicatum* after it produced a dense canopy at the water surface, and before *N. lutea* seedlings had achieved sufficient petiole elongation in deeper water could have been a factor in poor survival of *N. lutea* in these conditions. Al Hamdani and Francko (1992) found the optimum light intensity for seedling petiole elongation to be around ¼ of full sun. In lower underwater light conditions, rate of elongation is slowed. Madsen (1991) characterized the light environment under a *M. spicatum* canopy as intensely shaded. *Nelumbo lutea* can exhibit competitive suppression against *M. spicatum* when given the opportunity to become well established, but may not have the opportunity to establish from seedlings in an already developed *M. spicatum* canopy, particularly at depths approaching 1 m.

Community manipulation by species introduction requires a thorough understanding of successful establishment techniques. *Myriophyllum spicatum* established well at all depths, and exhibited the ability to spread among the containers successfully. *Nelumbo lutea,* however, could not establish successfully when planted as

seedlings in deeper water. Also, since the species spreads primarily by stoloniferous growth, its spread was hampered by the physical constraints of the containers. Tuber production was limited to the edges of the containers, and further radial spread of second-year *N. lutea* plants was limited. In a situation where *N. lutea* is allowed unrestrained spread, competitive effects should be enhanced.

Survival and growth of seedlings of *N. lutea* was limited to shallow water conditions. Various resolutions of depth observations in the three studies (0.1 meter, 0.2 meter or 0.5 meter) led to similar conclusions that seedling success was dependent on depth of planting. Although some variability was observed, *N. lutea* seedlings did not survive and grow at depths of one meter or more.

In a study on effects of water level fluctuation in Japan, *Nelumbo nucifera*, the other species of *Nelumbo*, was reported to grow and successfully regenerate (from tubers) in depths of 3 meters (Nohara and Tsuchiya, 1990). In the community dynamics pond study we observed second-season *N. lutea* plants successfully expanding and competing in depths greater than one meter after initial establishment in shallower water. A key to community manipulation would be to plant seedlings in shallow water, and expect competitive results in the second season of growth. These findings indicate a potential for using *N. lutea* as an aquatic ecosystem management tool to negatively impact a *M. spicatum* population through the deliberate introduction of a *N. lutea* population. In this context, techniques to assure successful establishment of *N. lutea* seedlings are imperative. *Nelumbo lutea* populations produce abundant seeds, which germinate readily upon physical scarification (DeGroft and Francko, 1996). Plant communities are unlikely

to be impacted from some seed harvesting; asexual propagation is apparently a primary mechanism for expansion of a *N. lutea* community.

Nelumbo lutea seedlings exhibit high success of growth and leaf production in a shallow environment with adequate light. Given a chance for initial establishment, *N. lutea* can move into deeper environments through rhizome production and radial spread. Successful establishment of *N. lutea* communities, however, requires short-term survival of seedlings.

CHAPTER 6.

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Table 1. Percent survival, petiole production, and mean shoot:root ratio of surviving seedlings (±SE) after 6 weeks of growth at each experimental light treatment. Capital letters denote significant differences (alpha 0.05, ANOVA).

Light Treatment	Percent Survival	Mean number of petioles of survivors	Mean shoot/root ratio
7%	100	3.17 ± .20 A	3.07 ± .34 A
10%	94	4.42 ± .20 B	2.27 ± .20 B
24%	100	4.06 ± .24 B	1.12 ± .08 C
100%	100	4.75 ± .31 B	0.65 ± .06 D

Table 2. Percent survival, petiole production, and mean shoot:root ratio of surviving seedlings (± SE) after 6 weeks of growth at each experimental planting depth.

Depth of Planting	Percent survival	Mean number of petioles of survivors	Significance (alpha=0.05, ANOVA)	Mean shoot:root ratio	Significance (alpha=0.05, ANOVA)
0.5 meters	100	4.75± 0.32	A	0.65± 0.06	A
1.0 meters	56	4.33± 0.52	A	2.82± 1.09	B
1.5 meters	12.5	3.0± 1.0	A	7.94± 2.08	C

Table 3. Average light level at *N. lutea* pot surface compared to light available at water surface and above water surface.

Source	Planting Depth	% full sun at pot level	% full sun subsurface	% full sun above surface	% survival
Depth study	0.5 m	41	49	100	100
	1.0 m	26	49.5	100	56
	1.5 m	19	52	100	13
Light study	0.45 m	19	24	~24	100
	0.45 m	8	9.3	~10	94
	0.45 m	6	6.7	~7	100

Figure 1. Above and below ground biomass of *N. lutea* versus percent light treatment at 3 and 6 week harvests ±SE (n=18). Lower case letters and roman numerals denote significant differences between 3rd and 6th week growth in above or below ground biomass, respectively. Capital letters denote differences between 3rd and 6th week growth in total biomass: (t-test, $p<0.05$).

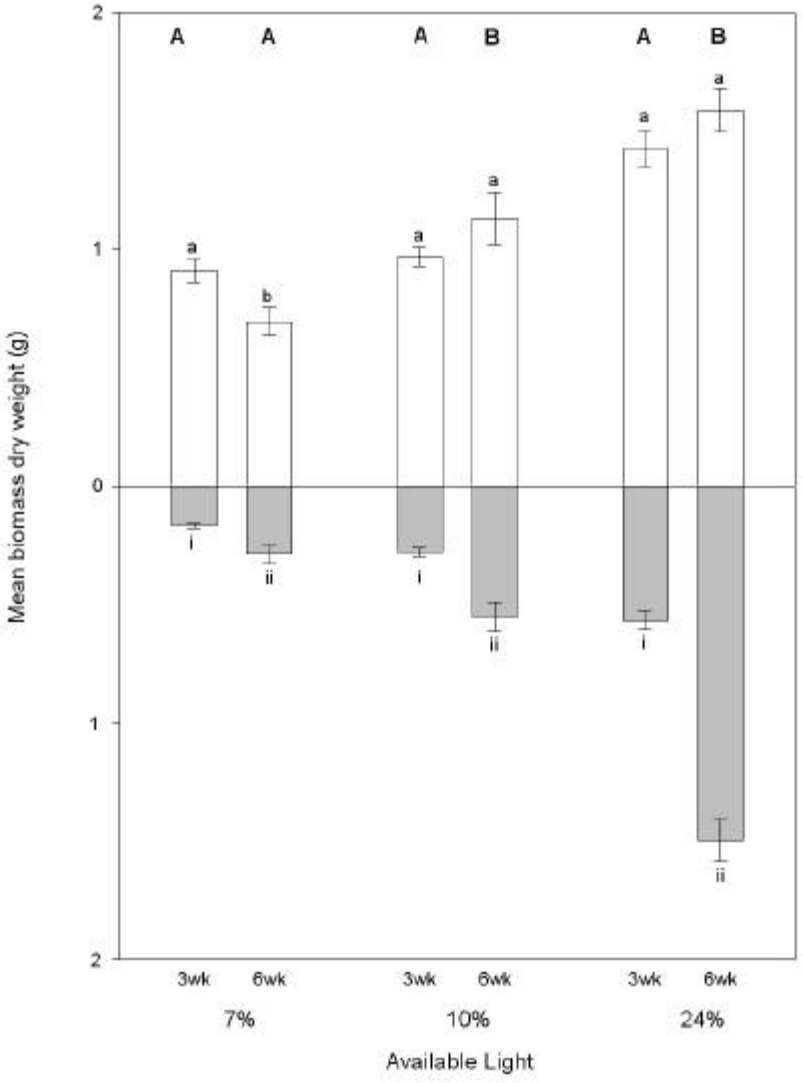


Figure 2. Total biomass of *N. lutea* versus percent light available at the water surface at 6 week harvest (n=18 for 7, 10, and 24%, n=16 at 100%. Capital letters denote significant differences: (Kruskal-Wallis non-parametric ANOVA, $p<0.01$).

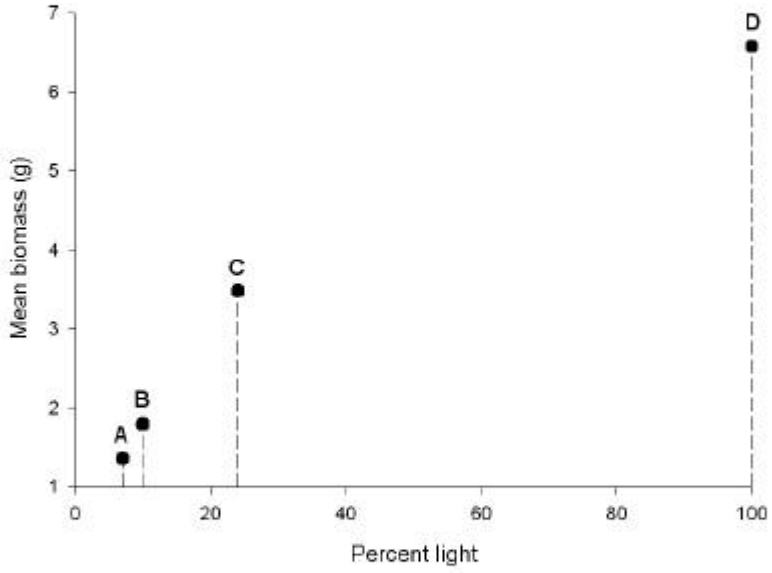


Figure 3. Above and below ground biomass of surviving *N. lutea* plants versus depth of planting (mean ± SE): N=the number of surviving plants; original plantings were 8 per tank by 2 tanks. Lower case letters and roman numerals denote significant differences among above and below ground biomass, respectively, and capital letters denote differences among total biomass measurements:
(Kruskal-Wallis non-parametric ANOVA, $p<0.01$).

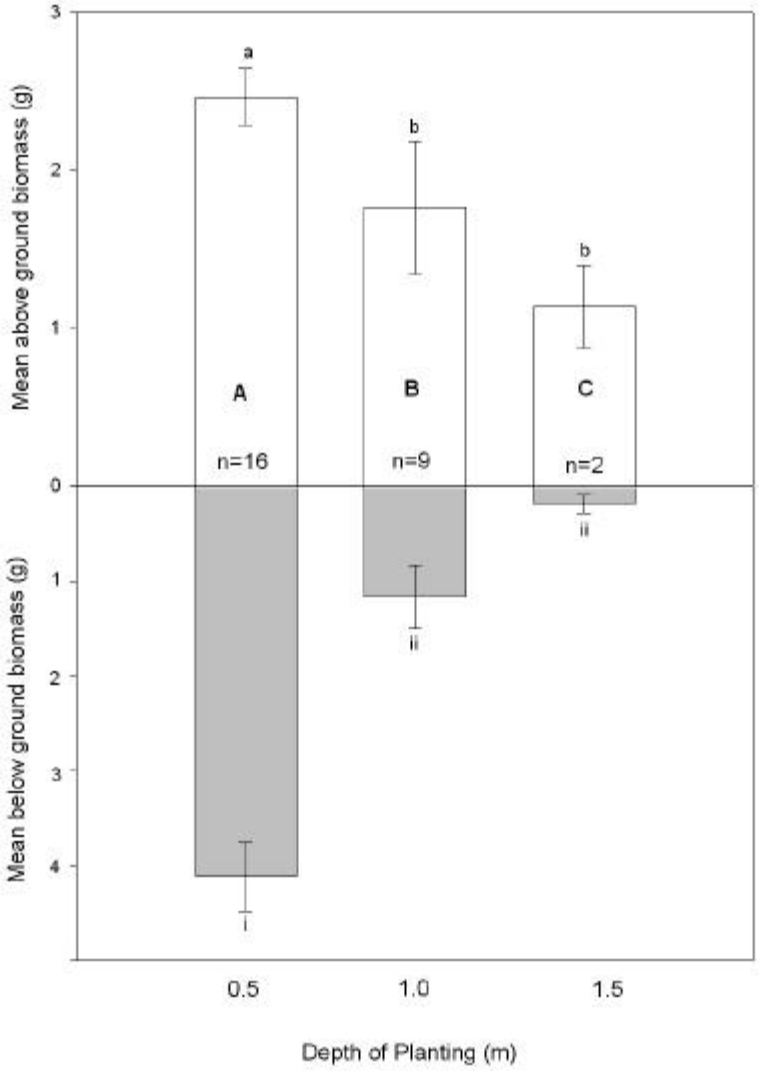
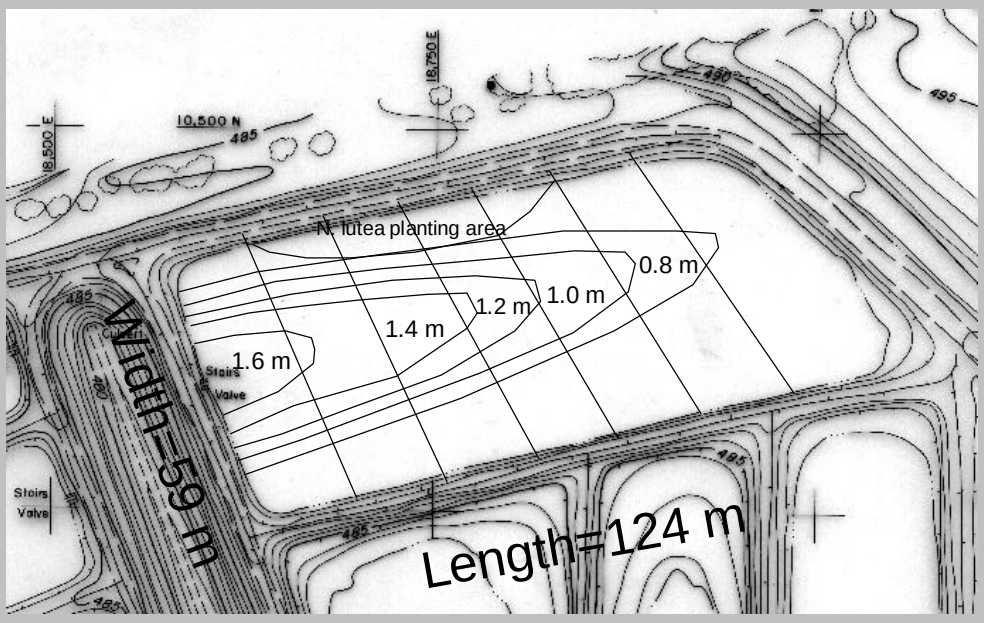


Figure 4. Community dynamics study pond. Lines across pond denote approximate transect locations. Depth contours are noted.



Map from Texas Parks and Wildlife Dept.

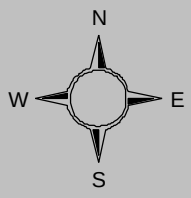


Figure 5. Frequency of occurence of *N. lutea* and *M. spicatum* in pond transects as percent of total transect points observed. At November monitoring, *N. lutea* was senescent, and no plants were observed.

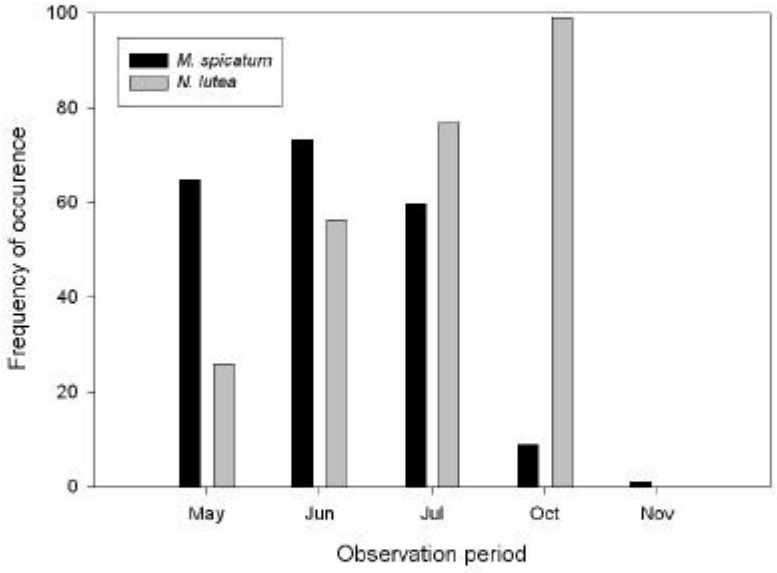


Figure 6. Frequency of occurence of *N. lutea* and *M. spicatum* as percent of total transect points observed in shallow and deep areas along pond transects.

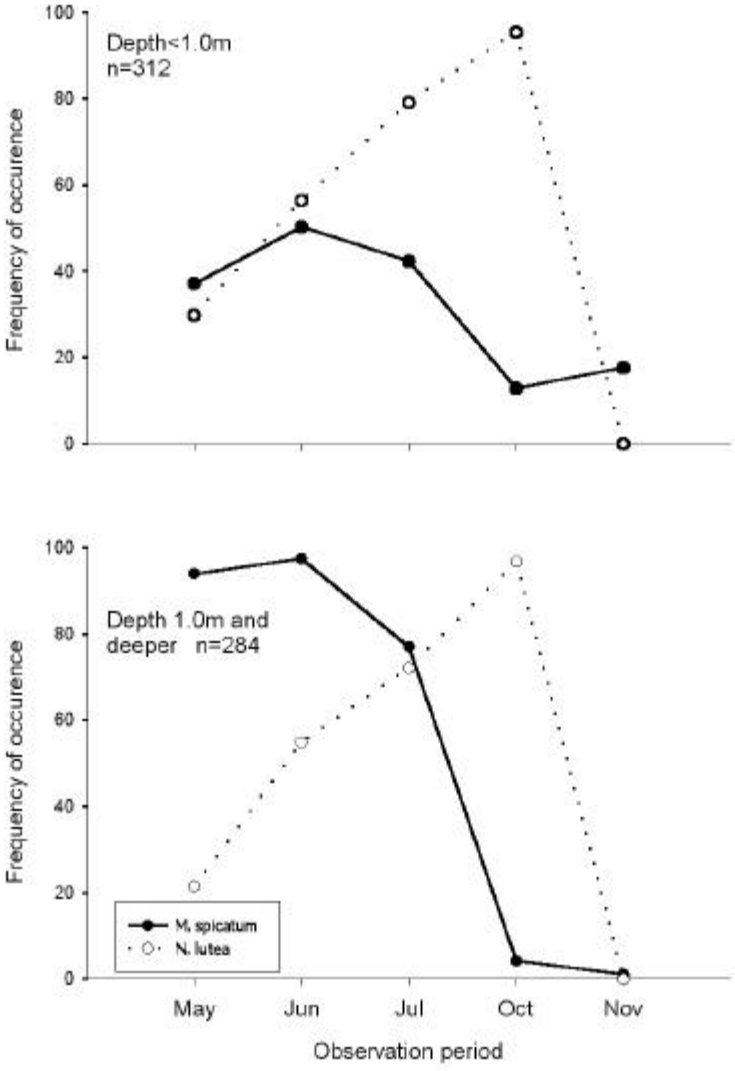


Figure 7.
A. Seven planting areas partitioned in each pool, one propagule (or none) in each area.

B. Pool layout in pond bottom, with mean depths of 4 depth blocks delineated, and planting scheme in each pool showing combinations of *N. lutea* and *M. spicatum*.

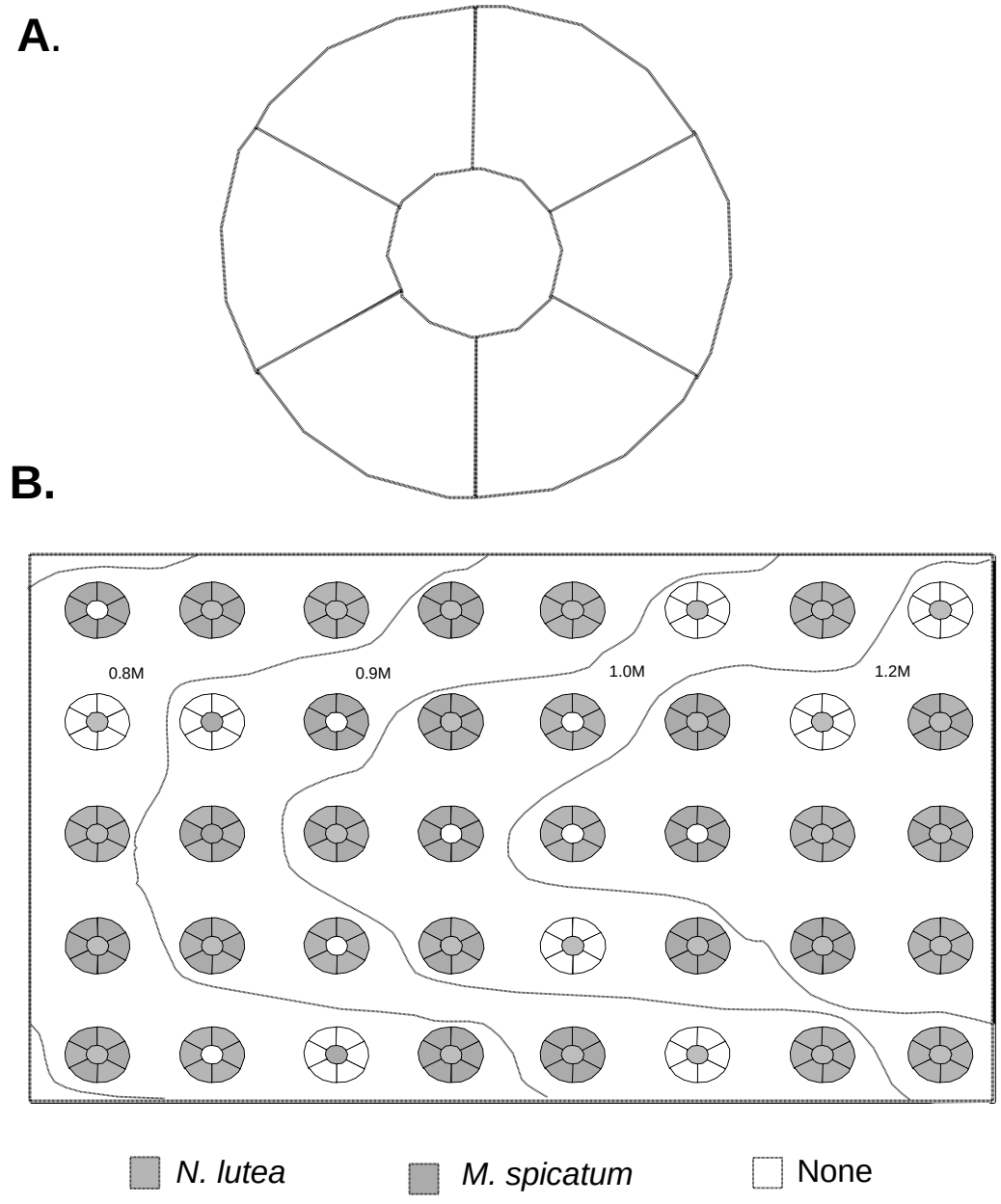


Figure 8. Effect of depth of planting of *N. lutea* on its percent cover after 2 seasons of growth. *N. lutea* planted in shallow water are marked by circles; those planted in deeper water are marked with triangles. A and B denote significantly different groups.

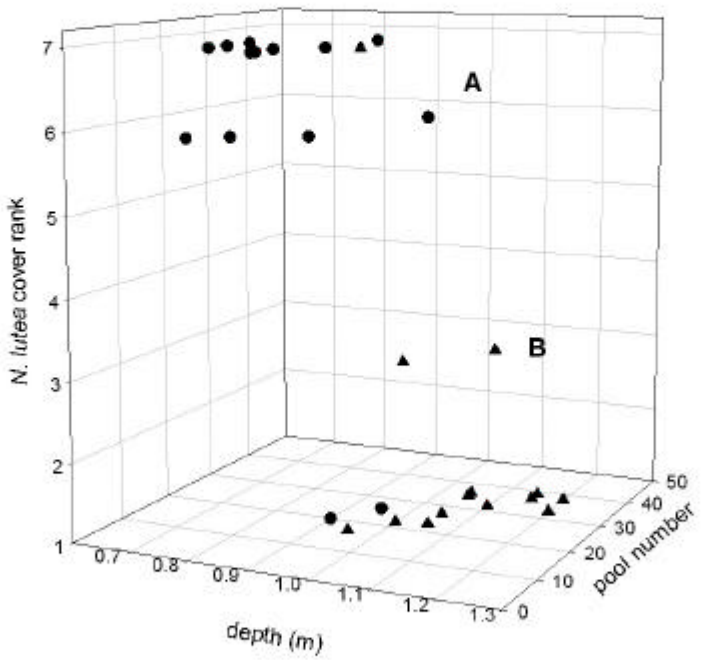
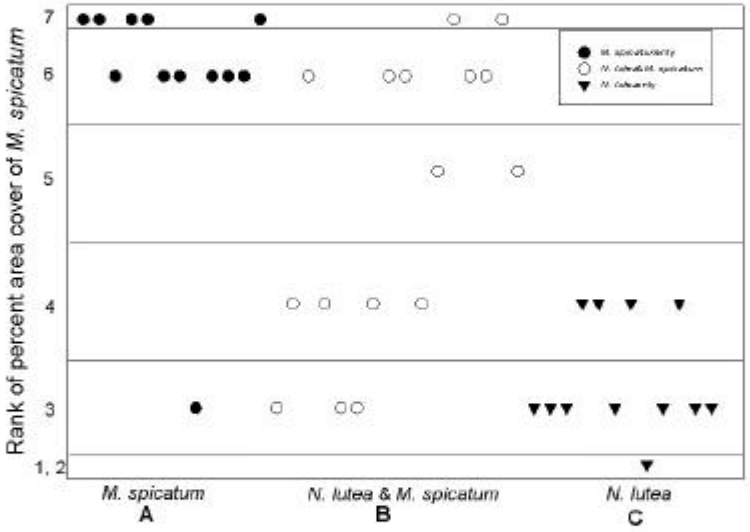


Figure 9. Ranking of percent cover of *M. spicatum* after 2 growing seasons as affected by *N. lutea* planting. A, B, and C are significantly different groups (alpha=0.05, SNK)



Planting scheme of *N. lutea* and *M. spicatum*

Figure 10. Effect of depth of planting on *M. spicatum* on its percent cover after 2 seasons of growth. No significant differences in plant establishment due to planting depth are observed.

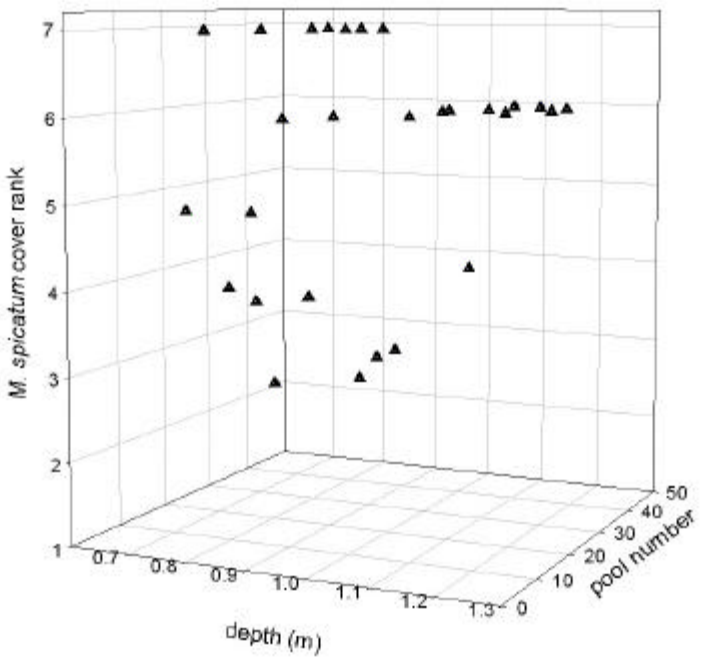


Figure 11. Competitive effect of *N. lutea* on the establishment of *M. spicatum* after 2 growing seasons. Pools which were not planted with *N. lutea* are marked by circles. Pools where *N. lutea* was planted in shallow water are marked with triangles. Where *N. lutea* became well-established, *M. spicatum* coverage was suppressed.